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FULL ARTICLE

Evaluation of corneal structures in myopic eyes more than twenty-two years after photorefractive keratectomy

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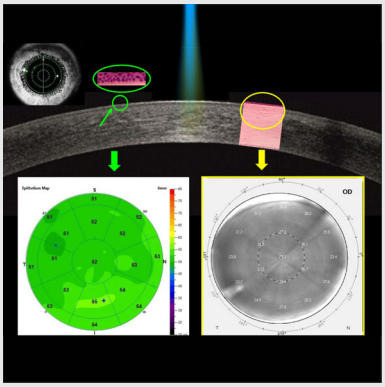
Abstract



The aim of this study is to evaluate corneal epithelial thickness (CET), corneal densitometry (CD) in 84 myopic eyes (57 patients) more than 22 years after photorefractive keratectomy, using anterior segment-optical coherence tomography (AS-OCT) and Scheimpflug imaging system. The CET was significantly higher in all operated eyes than in unoperated eyes in central sector. A statistically significant increase in CD in corneal anterior layer of central sector was shown in groups of operated eyes with greater ablation depth respect to unoperated eyes. While there was no significant difference in CD between the operated eyes groups with lower ablation depth and unoperated eyes. A significant trend toward higher values in anterior CD with deeper ablations in central sector was found. These noninvasive imaging techniques allow to better understand the corneal remodeling process after photoablation and to monitor the patients over time.

KEYWORDS

anterior segment-optical coherence tomography, corneal densitometry, corneal epithelial thickness, photorefractive keratectomy, scheimpflug imaging system

1 | INTRODUCTION

Photorefractive keratectomy (PRK) has been widely used in the treatment of myopia since the arrival of excimer laser technology just over 22 years ago.[1–6]

The structural remodeling of the cornea that occurs after PRK, involving epitelial hyperplasia and keratocytes activation.[7–9]

Newly developed diagnostic techniques, such as anterior-segment optical coherence tomography (AS-OCT) and anterior eye segment tomography (Oculus Pentacam HR type 70 900) with a rotating Scheimpflug camera system, have allowed to objectively measure preoperative stages and to monitor postoperative corneal changes after refractive surgery.[8–10] They are noncontact tools and reproducible methods that offer a more precise and faster

mapping of the corneal epithelium thickness (CET) and corneal densitometry (CD).[11, 12] AS-OCT represents a useful method to monitoring the alterations of CET covering the 6 mm diameter area.[13] The CD is evaluated on the intensity of backscattering light in different corneal sectors.[14, 15] Pentacam Scheimpflug system allows to measure CD not only at the central apex but also at peripheral corneal areas outside the treatment sector.[8]

To our knowledge, there are no previous reports of CET, CD in myopic patients with long-term follow-up after PRK. The purpose of this retrospective study was to value the changes of these parameters in myopic patients more than 22 years after PRK using the AS-OCT and Pentacam Scheimpflug system, respectively.

2 | MATERIALS AND METHODS

A retrospective review of clinical charts of patients that underwent PRK from January 1992 to December 1997 was conducted.[2, 6, 16, 17] Data were available on 737 myopic eyes of 612 patients. Exclusion criteria included current or past ocular pathology, corneal or intraocular surgery, ocular trauma (excluding PRK), corneal epithelial dystrophies, corneal opacities (haze), dry eye disorder, ocular or systemic diseases (in particular diabetes and autoimmune diseases) that may affect the anterior segment. Overall, 84 eyes (57 patients) were enrolled: 30 patients underwent PRK in one eye (30 eyes) while 27 patients in both eyes (54 eyes). Patients with one operated eyes were divided according to the depth of ablation in two groups: group 1 consists of 15 eyes that underwent 10 to 75 μm ablation depth and their 15 unoperated fellow eyes, group 2 consists of 15 eyes that underwent 76 to 120 μm ablation depth and their 15 unoperated fellow eyes. Moreover, we analyzed the whole group of 84 operated eyes that was divided according to the same ablation depth in group A (55 eyes) and group B (29 eyes). The groups A and B were compared with the unoperated eyes, represented by the fellow eyes of the groups 1 and 2 (30 eyes).

All patients underwent best corrected visual acuity, Goldman applanation tonometry, anterior segment slit-lamp biomicroscopy, tear film breakup time (TBUT), Schirmer I test, CD analysis using Pentacam Scheimplug device (Oculus Optikgeräte GmbH, Wetzlar, Germany) and measurements of CET using Fourier-Domain AS-OCT system RTVue (Optovue Inc, Fremont, California).

The investigational review board of the University of Naples "Federico II" reviewed the protocol and approved the study and all investigations adhered to the tenets of the Declaration of Helsinki. Written informed consents were obtained from the patients enrolled in the study.

2.1 | Surgical technique

For PRK, patients were given topical anesthesia by oxybuprocaine chloride eye drops. The lids were opened with a speculum and the epithelium was debrided with a Desmarres blade. A 193 nm excimer laser (Aesculap, Meditec, Jena, Germany) operating in a scanning mode with a 7 × 1-mm slit, with a repetition rate of 20 Hz, and the energy rate ranged from 300 to 500 mL/cm^2 was used. All eyes were treated using a mask with a vacuum fixation device and with an iris diaphragm that initiated treatments with a diameter of 5 mm and progressively closed, producing a flattening of the central cornea. Afterwards, a new mask was manufactured with an iris diaphragm that stars from 6 mm in diameter for treatments less than 6.5 diopters and 7 mm in diameter for higher treatments in which, in addition to the standard Munnerlyn algorithm used centrally, a transition zone outside this area was used. At the end of all surgical procedures, preservative-free gentamycin eye drops were applied and the eye was patched. The same eye drops were administered four times daily until re-epithelization was complete, and no corticosteroid eye drops were used.[2]

2.2 | Anterior segment optical coherence tomography system

The Fourier-Domain AS-OCT system RTVue (Optovue Inc) with a cornea anterior module long adapter lens and software version A6 (9.0.27), was used to measure CET. Data output included CET maps corresponding to an area 6 mm in diameter. The settings were: L-Cam lens, 8 meridional B-scan per acquisition, consisting of 1024 A-scan each with 5 μm axial resolution. Each CET map was divided into 17 sectors: a central sector 0 to 2 mm in diameter, 8 paracentral sectors from 2 to 5 mm in diameter and 8 peripheral sectors from 5 to 6 mm in diameter.[13]

2.3 | Scheimpflug densitometry

CD analysis was provided as an add-on to the standard software of the Pentacam HR type 70 900 with a rotating Scheimpflug camera (Oculus Optikgeräte GmbH). The measurement protocol took a series of 25 images (1003 × 520 pixels) over different meridians with a uniform blue light source. The acquisition protocol took approximately 2 seconds to complete. In the analysis, the program automatically located the corneal apex and analyzed an area around it with a 12 mm diameter. The output was expressed in grayscale units (GSU). The GSU

defined a minimum light scatter of 0 (maximum transparency) and maximum light scatter of 100 (minimum transparency).

The 12-mm-diameter corneal area is subdivided into four concentric radial sectors: a central sector was 2 mm in diameter and was centered on the apex, a first annulus

TABLE 1 Demographic and clinical information of PRK patients

	Total group	Group A	Group B	Group 1	Group 2
Eyes (n.)	84	55	29	15	15
Patients (n.)	57	35	22	15	15
Age (years)	58.52 ± 10.42	54.72 ± 10.36	62.72 ± 7.60	60 ± 12.58	62.73 ± 8.53
Sex (female/male)	34/23	22/13	12/10	8/7	10/5
Mean duration of follow-up (years)	24.1 ± 1.7	–	–	–	–
Deep of ablation (μm)	64.01 ± 26.24	48.76 ± 17.87	92.93 ± 10.17	54.60 ± 13.73	97.06 ± 9.69
Unoperated fellow eyes (n)	30	(–)	(–)	15	15

Note: Data are expressed as mean ± SD.
Abbreviation: PRK: Photorefractive Keratectomy.

TABLE 2 Differences in corneal epithelial thickness between 30 operated eyes divided according to ablation depth and unoperated fellow eyes

	Corneal epithelial thickness parameters, μm						
	Group 1			Group 2			
	Operated eyes	Unoperated fellow eyes	P^a	Operated eyes	Unoperated fellow eyes	P^a	Operated eyes group 1 vs group 2 P^b
Central sector	62.11 ± 6.64	54.86 ± 6.08	.025	64.46 ± 7.19	56.20 ± 5.26	.021	0.503
Paracentral corneal epithelium thickness							
Superior sector	56.88 ± 5.23	51.93 ± 4.69	.142	57.69 ± 6.66	52.53 ± 3.88	.116	0.946
Superior nasal sector	56.66 ± 5.72	52.26 ± 4.13	.109	58.23 ± 6.01	53.40 ± 3.69	.087	0.481
Superior temporal sector	57.66 ± 4.18	52.33 ± 5.40	.097	58.15 ± 5.95	52.86 ± 3.97	.077	0.893
Inferior sector	61.44 ± 5.98	55.26 ± 5.18	.138	60.76 ± 6.66	55.80 ± 4.12	.058	0.640
Inferior nasal sector	58.11 ± 5.57	54.80 ± 4.63	.189	59.69 ± 7.06	56.26 ± 4.46	.221	0.593
Inferior temporal sector	61.22 ± 5.82	55.26 ± 5.72	.138	60.84 ± 5.42	55.40 ± 5.97	.080	0.867
Nasal sector	56.33 ± 5.09	53.80 ± 4.36	.235	59.23 ± 6.90	55.26 ± 5.48	.169	0.314
Temporal sector	60 ± 5.43	53.73 ± 5.95	.123	59.84 ± 6.01	54.73 ± 5.56	.116	0.688
Midperipheral corneal epithelial thickness							
Superior sector	51.44 ± 4.77	50.33 ± 4.22	.796	48.69 ± 6.08	47.93 ± 4.96	.916	0.240
Superior nasal sector	52.44 ± 3.81	51.53 ± 3.70	.256	48.30 ± 4.62	50.06 ± 4.63	.400	0.077
Superior temporal sector	51.55 ± 4.44	50.66 ± 4.15	.988	49.92 ± 5.55	47.60 ± 6.57	.666	0.568
Inferior sector	56.22 ± 4.79	54.06 ± 3.08	.171	54.38 ± 5.10	54.80 ± 3.85	.998	0.421
Inferior nasal sector	54.44 ± 5	53.80 ± 3.14	.528	53.23 ± 5.62	54.33 ± 3.90	.843	0.545
Inferior temporal sector	56.88 ± 6.03	52.93 ± 3.51	.075	53.23 ± 5.63	53.40 ± 4.42	.999	0.255
Nasal sector	53 ± 3.50	52.73 ± 4	.400	51.07 ± 4.34	53.26 ± 4.51	.135	0.149
Temporal sector	54.44 ± 5.52	51.33 ± 3.69	.237	52.15 ± 5.45	51.93 ± 4.67	.944	0.383

Note: Data are expressed as mean ± SD.
[a]Wilcoxon signed-rank test, $P < .05$.
[b]Mann-Whitney test, $P < .05$.

extending from 2 mm to a 6-mm-diameter circle, a second annulus extending from 6 to 10 mm and the final annulus extending from 10 mm to a 12 mm- diameter circle. CD was evaluated, based on corneal depth, in anterior, central and posterior layers. The anterior layer corresponded to the anterior 120 μm and the posterior layer to the most posterior 60 μm of the cornea. The central corneal layer was defined by subtraction of the anterior and posterior layers from the total thickness.[15]

2.4 | Statistical analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (Version 20.0 for Windows; SPSS Inc, Chicago, Illinois), and with R (The R Project for Statistical Computing, https://www.r-project.org). Normality was tested by the Shapiro-Wilk test. The comparison of the CET and CD values between operated eyes of groups 1 and 2 and their unoperated fellow eyes was evaluated by the Wilcoxon signed-rank test, while the Mann-Whitney test was used to evaluate the differences in these parameters between the operated eyes of the groups 1 and 2, as well as to compare group A, group B and the unoperated eyes group. Before pooling together the eyes of monolaterally-operated patients and those of bilaterally operated patients, pairwise comparisons for the densitometry in all corneal sectors were conducted. Due to the non-normality of the data, Yuen's test[18] with 20% trimmed means was used, controlling for multiple comparisons with the false discovery rate (FDR). The Spearman's rank-order correlation was used to assess the relationship between the depth of ablation and the CD in different radial sectors in the anterior layer. A *P*-value <.05 was considered statistically significant.

3 | RESULTS

In this retrospective study 57 patients (mean age 58.52 ± 10.42 years) for a total of 84 myopic eyes (mean spherical equivalent −8.52 ± 5.25 diopters) were enrolled and

TABLE 3 Differences in corneal epithelial thickness among 86 operated eyes divided according to ablation depth and unoperated eyes

	Corneal epithelial thickness parameters, μm					
	Operated eyes (Group A)	Operated eyes (Group B)	Unoperated eyes	P^a	P^b	P^c
Central sector	60.26 ± 6.11	61.86 ± 7.30	55.53 ± 5.63	.001	.002	.446
Paracentral corneal epithelium thickness						
Superior sector	55.33 ± 5.42	56.95 ± 6.01	52.23 ± 4.24	.019	.003	.441
Superior nasal sector	56.07 ± 4.91	58.26 ± 4.68	52.83 ± 3.89	.009	<.001	.122
Superior temporal sector	55.69 ± 5.31	57.21 ± 6.23	52.60 ± 4.67	.016	.010	.346
Inferior sector	59.52 ± 5.85	60 ± 5.47	55.53 ± 4.61	.004	.003	.626
Inferior nasal sector	58.78 ± 6.30	59.17 ± 6.01	55.53 ± 4.53	.029	.016	.550
Inferior Temporal sector	59.26 ± 5.52	59.65 ± 4.72	55.53 ± 5.74	.006	.005	.773
Nasal sector	57.52 ± 4.92	58.65 ± 5.75	54.53 ± 4.92	.017	.010	.479
Temporal sector	57.30 ± 5.32	58.52 ± 5.57	54.23 ± 5.68	.035	.019	.554
Midperipheral corneal epithelial thickness						
Superior sector	50.97 ± 4.96	49.91 ± 5.86	49.13 ± 4.68	.249	.808	.425
Superior nasal sector	52.66 ± 4.44	50.60 ± 5.81	50.80 ± 4.18	.100	.455	.028
Superior temporal sector	50.69 ± 4.85	50.17 ± 5.18	49.13 ± 5.62	.630	.857	.582
Inferior sector	56.57 ± 5.71	55.47 ± 5.59	54.43 ± 3.45	.188	.565	.611
Inferior nasal sector	55.73 ± 5.96	54.13 ± 5.37	54.06 ± 3.49	.506	.794	.346
Inferior Temporal sector	56.14 ± 5.65	53.95 ± 5.36	53.16 ± 3.93	.056	.808	.167
Nasal sector	53.97 ± 4.30	52.26 ± 4.64	53 ± 4.20	.403	.476	.130
Temporal sector	52.80 ± 5.86	51.86 ± 4.61	51.63 ± 4.16	.651	.921	.810

Note: Data are expressed as mean ± SD. Mann-Whitney test, $P < .05$.
[a]Group A vs unoperated eyes group.
[b]Group B vs unoperated eyes group.
[c]Group A vs group B.

were divided in two groups according to the depth of ablation: groups A (55 eyes, mean age 54.72 ± 10.36 years) and group B (29 eyes, mean age 62.72 ± 7.60 years). The 30 patients that underwent PRK in a single eye (30 eyes) were divided in two groups according to the depth of ablation: group 1 (15 operated eyes and 15 fellow eyes, mean age 60 ± 12.58 years), group 2 (15 operated eyes and 15 fellow eyes, mean age 62.73 ± 8.53 years).

No significant difference in term of gender was found between the groups A and B ($P = .362$) and between groups 1 and 2 ($P = .368$). A statistically significant reduction in age was found in group A compared to unoperated eyes ($P = .010$) and group B ($P = .002$), while no statistically significant difference was shown between group B and unoperated eyes ($P = .425$). The age was similar between operated eyes of the groups 1 and 2 ($P = .256$). The mean duration of follow-up in this study was 24.1 ± 1.7 years. Table 1 lists the demographic and clinical information of each PRK group.

3.1 | CET results

A significant increase in CET only in the central sector was found in operated eyes (groups 1 and 2) respect to

TABLE 4 Differences in corneal densitometry between 30 operated divided according to ablation depth and unoperated fellow eyes

	Corneal densitometry (GSU)						
	Group 1			Group 2			
	Operated eyes	Unoperated fellow eyes	P^a	Operated eyes	Unoperated fellow eyes	P^a	Operated eyes Group 1 vs Group 2 P^b
Anterior layers							
0-2 mm	29.88 ± 4.31	27.84 ± 2.76	0.115	42.72 ± 11.78	26.65 ± 1.63	.001	.002
2-6 mm	25.32 ± 3.16	25.23 ± 3.08	0.727	31.88 ± 6.06	25.28 ± 2.91	.002	.001
6-10 mm	32.01 ± 8.24	33.85 ± 10.17	0.505	35.47 ± 11.48	35.43 ± 11.76	.383	.461
10-12 mm	39.74 ± 10.47	42.65 ± 11.60	0.576	39.89 ± 17.51	46.22 ± 13.96	.116	.652
Anterior total	30.41 ± 4.78	30.93 ± 5.63	0.972	36.14 ± 7.50	31.84 ± 6.37	.004	.021
Center layers							
0-2 mm	15.92 ± 1.57	16.09 ± 1.24	0.600	17.58 ± 2.20	15.94 ± 1.35	.035	.033
2-6 mm	14.96 ± 1.56	15.26 ± 1.71	0.328	16.61 ± 2.88	15.42 ± 1.89	.140	.081
6-10 mm	23.11 ± 7.23	24.27 ± 8.03	0.650	24.54 ± 7.38	24.82 ± 7.84	.572	.653
10-12 mm	27.21 ± 6.76	29.07 ± 7.42	0.638	27.17 ± 9.60	29.83 ± 6.63	.173	.621
Center total	19.59 ± 3.54	20.32 ± 4.13	0.576	20.92 ± 4.32	20.72 ± 4.08	.950	.461
Posterior layers							
0-2 mm	12.52 ± 1.23	13.20 ± 1.28	0.120	12.84 ± 1.47	12.69 ± 1.56	.851	.539
2-6 mm	12.18 ± 1.32	12.63 ± 1.68	0.286	13.36 ± 2.24	12.51 ± 1.67	.346	.161
6-10 mm	19.50 ± 5.76	20.23 ± 5.99	0.700	21.78 ± 5.87	21.05 ± 5.56	.683	.285
10-12 mm	23.49 ± 6.36	25.28 ± 6.59	0.279	24.10 ± 8.07	26.67 ± 5.63	.116	.983
Posterior total	16.30 ± 2.82	17 ± 3.33	0.506	17.61 ± 3.72	17.37 ± 3.14	.834	.436
Total 0-2 mm	19.44 ± 1.90	19.05 ± 1.68	0.387	24.38 ± 4.77	18.41 ± 1.26	.003	.004
Total 2-6 mm	17.49 ± 1.73	17.72 ± 2.11	0.506	20.62 ± 3.45	17.74 ± 2.08	.008	.013
Total 6-10 mm	24.87 ± 6.98	26.10 ± 8	0.675	27.28 ± 8.06	27.09 ± 8.19	.706	.436
Total 10-12 mm	30.15 ± 7.20	32.32 ± 8.12	0.133	30.37 ± 10.98	34.23 ± 7.70	.136	.914
Total	22.10 ± 3.51	22.76 ± 4.30	0.675	24.89 ± 4.97	23.31 ± 4.35	.124	.116

Note: Data are expressed as mean ± SD.
Abbreviation: GSU, grayscale units.
[a]Wilcoxon signed-rank test, $P < .05$.
[b]Mann-Whitney test, $P < .05$.

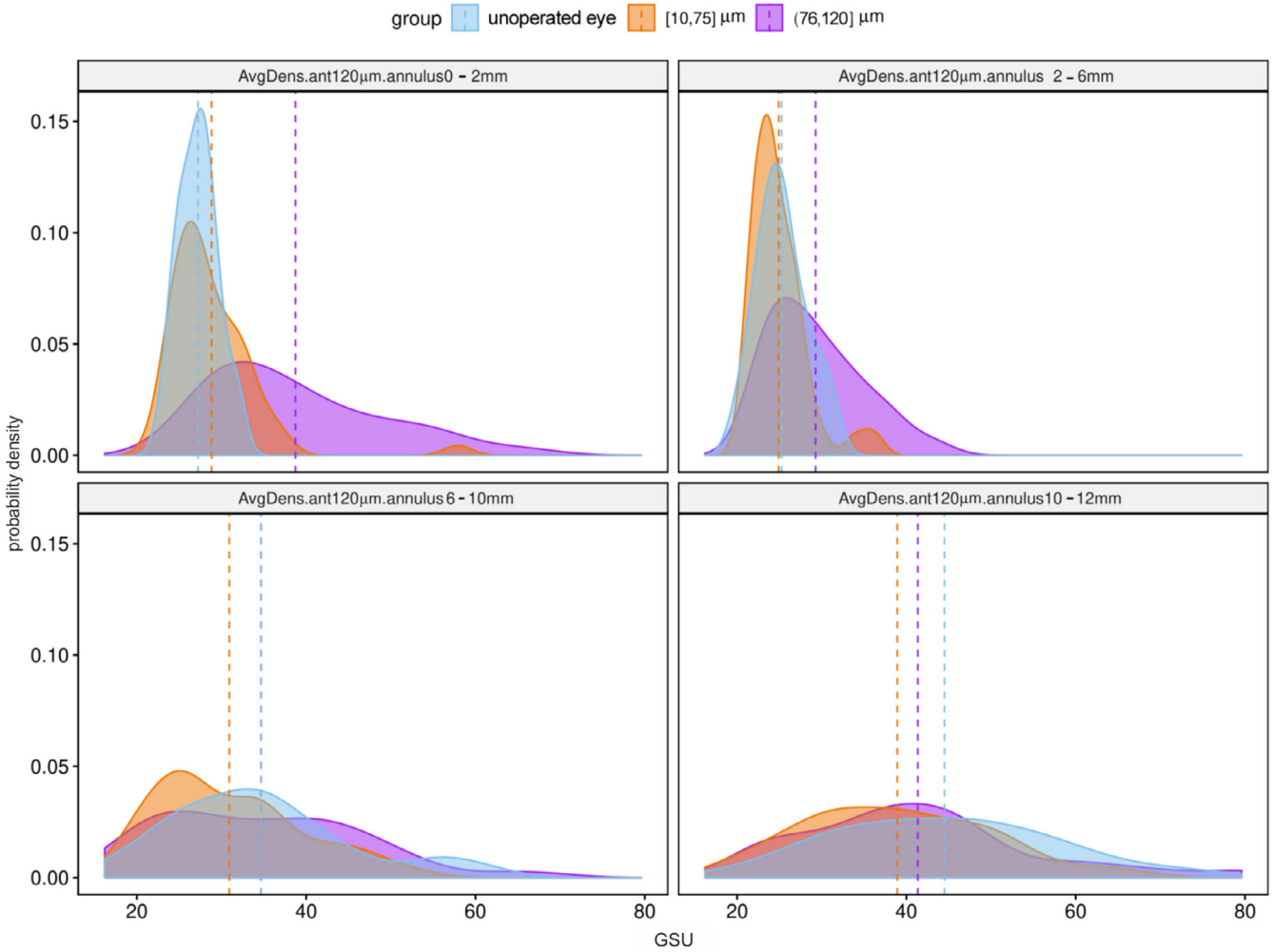


FIGURE 1 Empirical distribution (obtained with kernel density estimation) of the average corneal density in the four sectors of the anterior layer for group 1 operated eyes (orange), group 2 operated eyes (purple) and unoperated eyes (light blue). Dashed lines indicate the means. GSU, grayscale units

their unoperated fellow eyes ($P = .025$, $P = .021$). No significant difference was instead apparent in all sectors of CET between the operated eyes groups (Table 2).

Significant increase in CET was found in both groups A and B respect to unoperated eyes group in central and paracentral sectors. No significant difference in CET values was shown between operated eyes groups (Table 3).

3.2 | CD results

The CD values, evaluated according to depth, radial sector, showed no significant difference in group 1 between operated, and their unoperated fellow eyes. In group 2, the operated eyes showed significant greater CD values respect to their unoperated fellow eyes in the anterior layer (0-2 mm, 2-6 mm radial sectors; $P = .001$, $P = .002$), anterior total layer ($P = .004$), central layer (0-2 mm radial sector, $P = .035$) and total layer (0-2 mm and 2-6 mm radial sectors; $P = .003$, $P = .008$). Comparing the operated eyes of both groups, CD values were significantly higher in group 2 than group 1 in anterior layer (0-2 mm, 2-6 mm radial sectors; $P = .002$, $P = .001$), anterior total layer ($P = .021$), central layer (0-2 mm radial sector, $P = .033$) and total layer (0-2 mm and 2-6 mm radial sectors; $P = .004$, $P = .013$) (Table 4, Figure 1).

No significant difference was found (minimum of the adjusted $P = .184$) among the eyes whose fellow eye was not operated and the eyes whose fellow eye was operated. A trend similar to the one described above was found in all 84 operated eyes that showed a significant increase in CD values in the group B compared to the unoperated eyes group in anterior layer (0-2 mm and 2-6 mm radial sectors; $P < .001$, $P = .005$), central layer (0-2 mm

TABLE 5 Differences in corneal densitometry among 84 operated eyes divided according to ablation depth and unoperated eyes group

Corneal densitometry (GSU)						
	Operated eyes (Group A)	**Operated eyes (Group B)**	**Unoperated eyes**	P^a	P^b	P^c
Anterior layers						
0-2 mm	29.52 ± 7.84	38.72 ± 10.12	27.22 ± 2.28	.309	<.001	<.001
2-6 mm	24.91 ± 3.28	29.27 ± 5.51	25.25 ± 2.93	.346	.005	<.001
6-10 mm	30.91 ± 8.57	34.66 ± 11.55	34.67 ± 10.84	.126	.935	.232
10-12 mm	38.92 ± 11.03	41.34 ± 13.74	44.50 ± 12.76	.065	.270	.586
Anterior total	29.69 ± 4.77	34.43 ± 6.73	31.40 ± 5.92	.210	.114	.002
Center layers						
0-2 mm	15.82 ± 2.16	17.14 ± 1.91	16.01 ± 1.27	.206	.035	<.001
2–6 mm	14.52 ± 1.30	16.02 ± 2.51	15.34 ± 1.77	.057	.394	.006
6-10 mm	21.31 ± 7.03	24.53 ± 8.63	24.55 ± 7.78	.051	.774	.119
10-12 mm	25.70 ± 6.82	27.28 ± 7.49	29.47 ± 6.90	.027	.160	.467
Center Total	18.60 ± 3.42	20.67 ± 4.38	20.52 ± 4.03	.037	.915	.050
Posterior layers						
0-2 mm	12.55 ± 1.41	12.76 ± 1.21	12.94 ± 1.43	.210	.743	.361
2-6 mm	11.96 ± 1.33	12.99 ± 1.98	12.57 ± 1.64	.119	.450	.023
6-10 mm	18.02 ± 5.53	21.15 ± 6.60	20.65 ± 5.67	.025	.902	.042
10-12 mm	22.64 ± 6.56	24.26 ± 6.67	26 ± 6.03	.006	.277	.240
Posterior Total	15.58 ± 2.96	17.28 ± 3.64	17.20 ± 3.18	.018	.806	.048
Total 0-2 mm	19.29 ± 3.51	22.87 ± 4.01	18.72 ± 1.48	.886	<.001	<.001
Total 2-6 mm	17.14 ± 1.73	19.43 ± 3.08	17.73 ± 2.06	.175	.040	<.001
Total 6-10 mm	23.40 ± 6.85	26.78 ± 8,78	26.61 ± 7.96	.057	.909	.107
Total 10-12 mm	29.08 ± 7.49	30.95 ± 8.61	33.31 ± 7.81	.022	.242	.413
Total	21.29 ± 3.53	24.13 ± 4.73	23.05 ± 4.25	.068	.471	.010

Note: Data are expressed as mean ± SD. Mann-Whitney test, $P < .05$.
Abbreviation: GSU: grayscale units.
[a]Group A vs unoperated eyes group.
[b]Group B vs unoperated eyes group.
[c]Group A vs group B.

radial sector, $P = .035$), total layer 0-2 mm and 2-6 mm radial sectors ($P < .001$) and total layer ($P = .010$). Group A showed no statistically significant differences in CD respect to the unoperated eyes except for some peripheral radial sectors of the central, posterior layers and total layer. Comparing the operated eyes, CD values were higher in group B than group A in anterior layer (0-2 mm, 2-6 mm radial sectors), anterior total layer, central layer (0-2 mm, 2-6 mm radial sectors), central total layer, posterior layer (2-6 mm, 6-10 mm radial sectors), posterior total layer, total layer(0-2 mm and 2-6 mm radial sectors) (Table 5, Figure 2).

The Figures 1 and 2 showed the kernel-smoothed histogram of the average corneal densities in the four annulus sectors in operated eyes groups (with high and low depth ablation) and unoperated eyes. In each image, the three distributions were pretty overlapped in the external sectors (6-10 mm and 10-12 mm), while the difference was evident in the central sectors. Specially, in the 0-2 and 2-6 mm sectors, the distribution for the groups with greater ablation depth was different from the ones with lower ablation depth and unoperated eyes.

A significant relationship was found between the anterior CD in the 0-2 mm radial sector and the depth of ablation. This is visually confirmed in Figure 3, where the anterior CD was plotted against the depth of ablation. In the right part of Figure 3, where operated eyes were shown, a monotonic trend can be observed which was confirmed by Spearman's rank-order correlation ($r = 0.65$; $P < .001$). A cubic regression was performed ($R2 = 0.42$, $P < .001$).

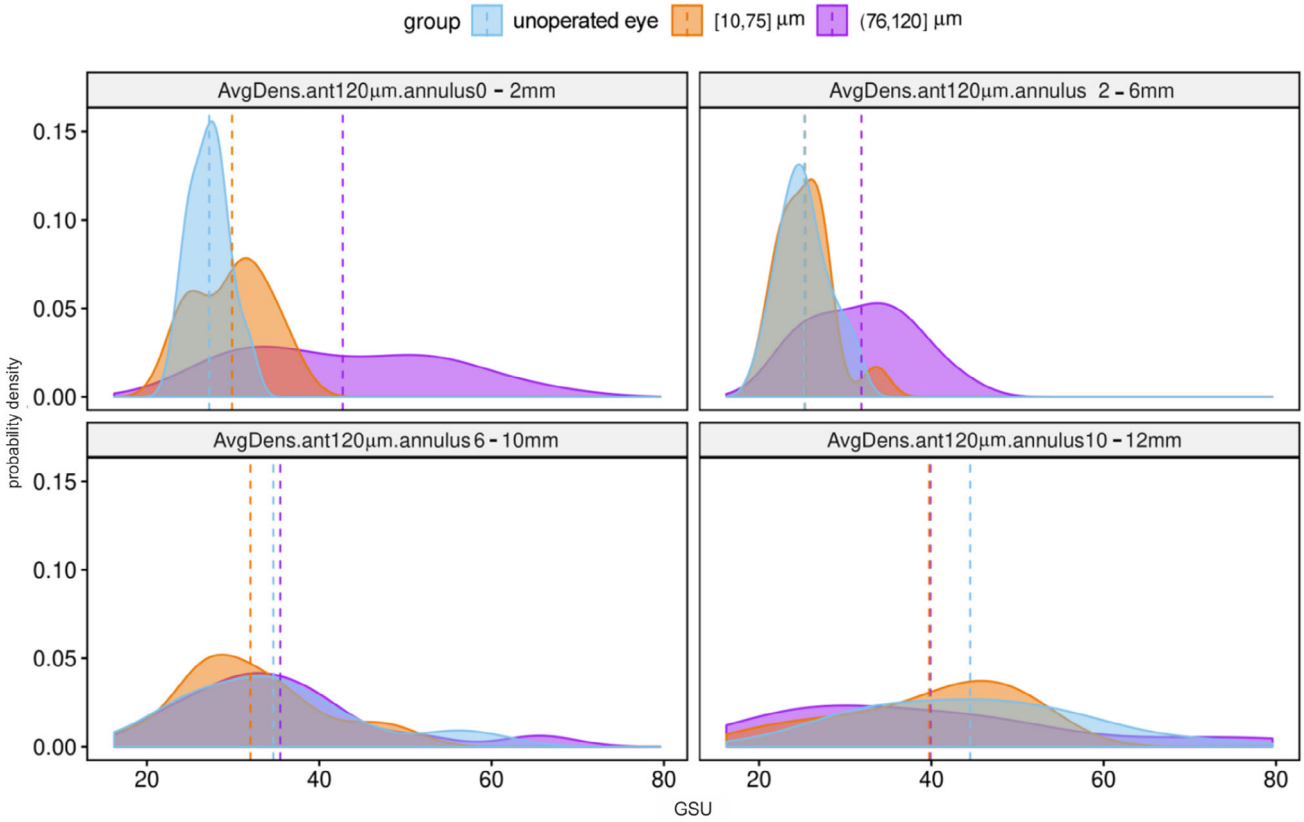


FIGURE 2 Empirical distribution (obtained with kernel density estimation) of the average corneal density in the four sectors of the anterior layer for group A eyes (orange), group B eyes (purple) and unoperated eyes (light blue). Dashed lines indicate the means. GSU, grayscale units

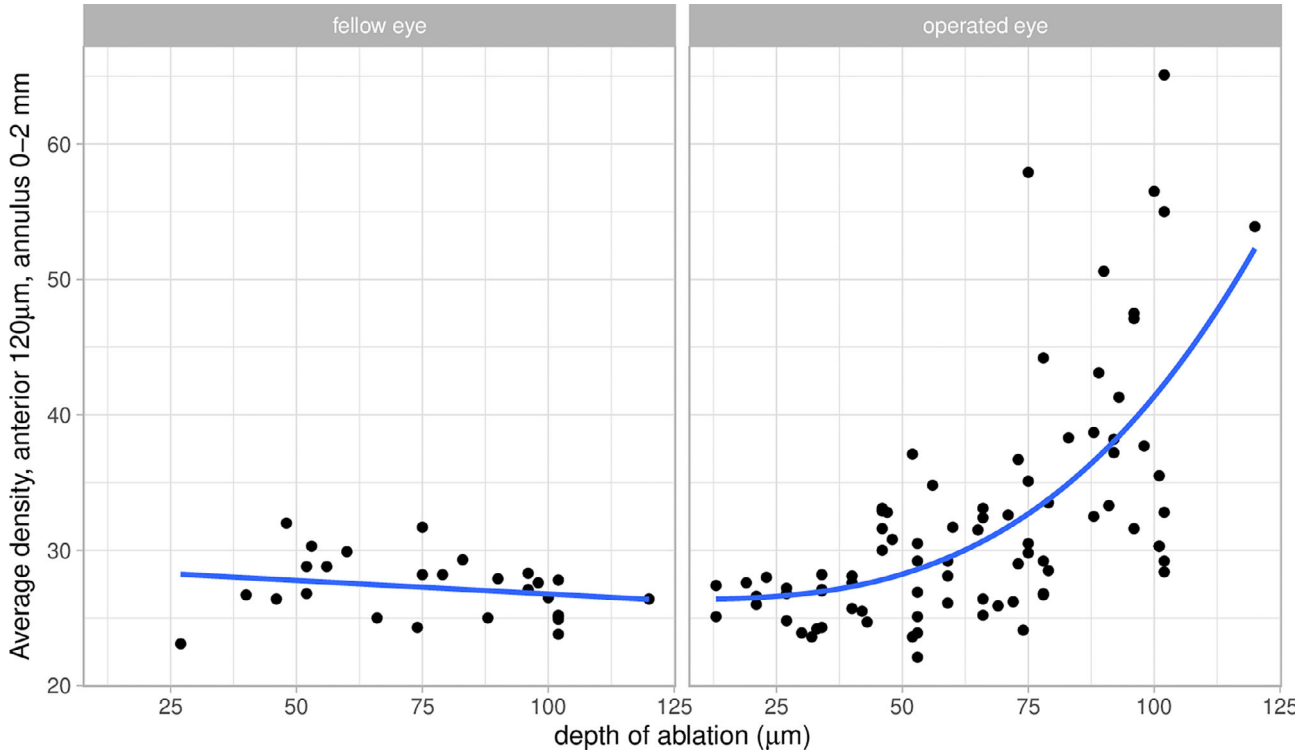


FIGURE 3 Anterior CD in the 0-2 mm radial sector (y-axis) vs. depth of ablation in µm (x-axis). The left part reports CD measurements for unoperated fellow eyes (in this case, the depth of ablation is the one of the corresponding operated eye) and the blue line is the linear regression line. The right part reports data for all 84 operated eyes and the blue curve is a cubic regression curve. CD, corneal densitometry

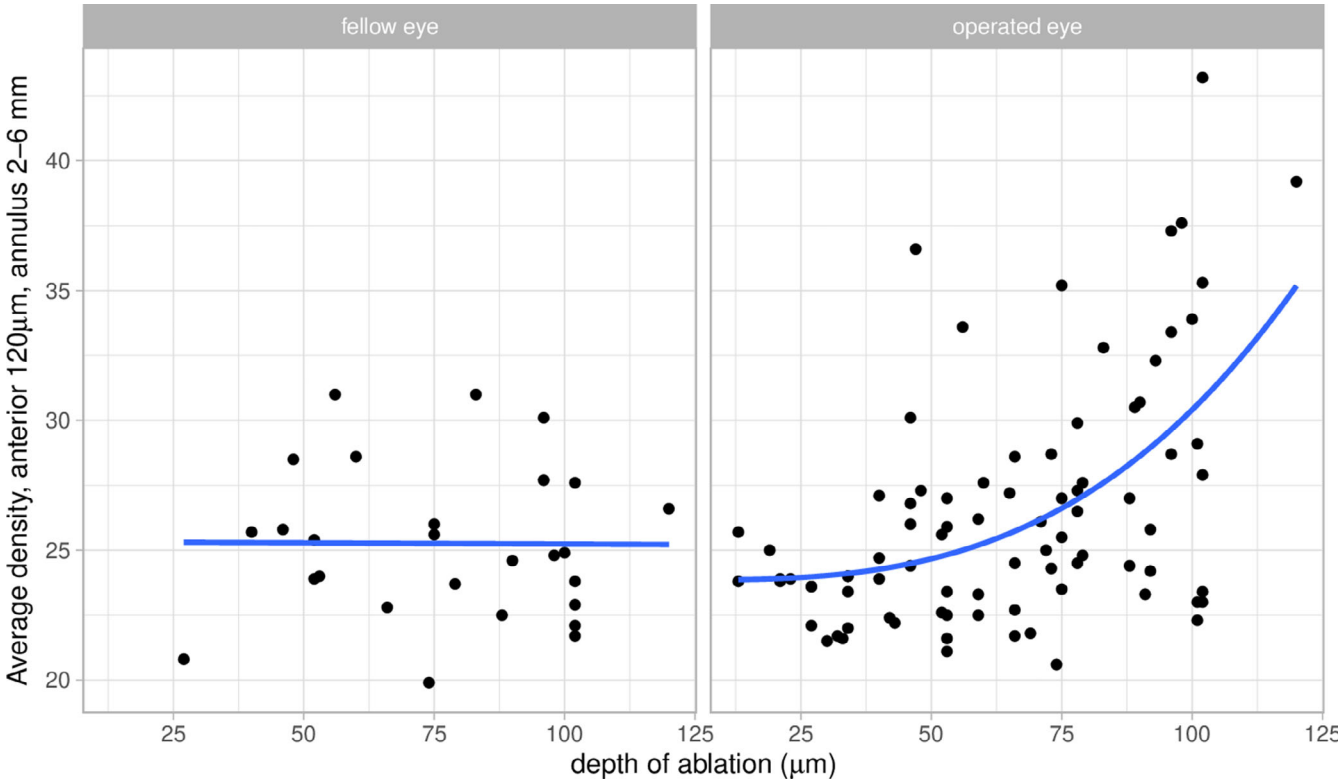


FIGURE 4 Anterior CD for the anterior 2-6 mm radial sector (y-axis) vs. depth of ablation in µm (x-axis). The left part reports CD measurements for unoperated fellow eyes (in this case, the depth of ablation is the one of the corresponding operated eye) and the blue line is the linear regression line. The right part reports data for all 84 operated eyes and the blue curve is a cubic regression curve. CD, corneal densitometry

The same reasoning as above applies, though with a larger variability, to the relationship between the anterior CD in the 2-6 mm radial sector and the depth of ablation (Figure 4). Also in this case, Spearman's rank-order correlation was used to assess the relationship ($r = 0.44$; $P < .001$) and a cubic regression was performed ($R2 = 0.28$, $P < .001$).

No significant correlation was found between the anterior CD and depth of ablation for the 6-10 mm, 10-12 mm radial sectors ($r = 0.13$, $P = .26$; $r = 0.06$, $P = .57$; respectively) that represent the peripheral areas that were not involved in photoablation.

4 | DISCUSSION

To our knowledge, this retrospective study is the first to evaluate CET and CD in eyes that underwent PRK after more than 22 years. The energy of PRK photoablation and the consequent wound-healing response modify the corneal structure. It has become increasingly clear that this response is both epithelial and stromal.[19] Thus, the interaction between stromal and epithelial healing should be considered to fully understand the corneal remodeling process and its clinical value.

The CET and CD represent important parameters that may to better understand the corneal changes after PRK. In our study, we found an increase in CET in central sector in all groups of operated eyes compared to unoperated eyes. This increase is due to the epithelial hyperplasia, as wound healing response after photoablation, confirming several studies that, using AS-OCT, showed a significant increase in CET after 6 months postoperatively.[9, 10, 20] Ivarsen et al, using scanning confocal microscopy, demonstrated that CET significantly increased after 3 years follow-up.[21] Gauthier et al found a significant increase in epithelial thickness between 13 and 37 months after PRK.[22] Further studies conducted by Erie and Patel, showed a significant increased CET 12 months after PRK and thereafter remained unchanged to 36 months and 7 years.[19, 23]

Regarding CD, we found that this parameter progressively raised with larger photoablation depths. Indeed the groups of the operated eyes with a greater photoablation depth, showed a significant increase in CD respect to those with lower photoablation depth and unoperated eyes in the corneal central sector of the anterior, central layers and total thickness. Conversely, no significant difference was found between operated eyes with lower photoablation depth and unoperated eyes.

These findings could be due to the response of the underlying stromal tissue to biological and chemical changes of the epithelial and stromal after photoablation. The epithelial injury triggers the release of proapoptotic cytokines that bind to receptors on keratocytes immediately beneath the wounded epithelium and starts a cascade of stromal healing events.[24–26] In the first 6 months, keratocytes density in the anterior stroma decrease by almost 40% and subsequently continues to fall by approximately 3.2% per year until to 5 years.[27] The remaining keratocytes surrounding the area of apoptotic loss undergone activation and begin to proliferate and to repopulate the treated area. The activated keratocytes, under the action of the growth factors such as transforming growth factor beta (TGFβ) and nerve growth factor (NGF) present in this area, transform into fibroblasts and later into myofibroblasts.[28, 29]

The main activity of repair-fibrocytes and myofibroblasts is the production of collagenases, proteases, metalloproteinases and extracellular matrix (ECM) components (type I, type III and type IV collagen, cellular fibronectin, tenascin and laminin) that play a crucial role in the corneal remodeling during the healing process[30, 31] and induce also a consequent relaxation of the peripheral collagen fibrils toward their limbal base stabilizing a new corneal curvature.[32–35]

Moreover, confocal microscope studies suggested that the activation of the keratocytes, after PRK, would seem to alter the expression of intracellular protein, namely crystallins. The alterations of these elements, that control the optical properties of the keratocytes, may contribute to the changes in corneal light backscattering.[36–38]

Therefore, the activation of keratocytes and the changes in stromal ECM seem to be responsible for increased corneal light backscattering.[31, 36, 39–41]

In our study, we also found a trend toward higher values in anterior CD with deeper ablations.

We hypothesized that the greater photoablation depth determines important changes in stromal layers that influences the corneal light backscattering and consequently the densitometry values.

These results are confirmed by the studies conducted by Moller-Pedersen et al that revealed, using confocal scanning microscopy, that the corneal light backscattering increased proportionally with greater stromal photoablation depth over the 12 months post PRK determining a raised keratocyte activation and myofibroblast transformation.[36, 42] Considering the studies that used the Scheimpflug imaging system, Boulze-Pankert et al showed a significant decrease in CD after 3 months post PRK,[43] Savini et al revealed that the laser in situ keratomileusis (LASIK) was followed by an increase in CD during the early postoperative period (3 months) and a reduction to baseline after 3 months.[44]

Also Cennamo et al have found an early increase and a subsequent reduction to similar values to baseline in anterior corneal optical density and refractive index during the 12 months post PRK suggesting a postoperative stromal remodeling as wound-healing response.[45]

Compared to our study, these reports analyzed the CD not considering the different depth of photoablation and for a short-term follow-up.

In our study the Figures 3 and 4 showed a similar trend in the distribution of CD for central sectors even if the comparison among the groups 1, 2 and unoperated fellow eyes turned to be slightly more evident respect to the comparison among the groups A, B and unoperated eyes. This behavior could be due to the possible influence of the age on CD[12, 15] that however it did not modify the trend of the different distribution in the various groups.

We also analyzed the CD of the peripheral annulus of the cornea demonstrating a different behavior. Unlike the comparison between operated eyes and their unoperated fellow eyes in group 1 that did not demonstrate differences in CD in any corneal sector, the group A showed lower values in CD respect to unoperated eyes in peripheral radial sectors.

These results could be due to the influence of the age on CD values, since the group A showed a statistically significant reduction in age respect to unoperated eyes. Infact several reports demonstrated the negative correlation between CD and age that increased progressively toward the peripheral radial sectors.[12, 15]

Our results revealed that the main changes in CET and CD after PRK were localized in the central sector that coincides with the area of PRK ablation, and in the anterior stroma that represents the main treated layer presenting the highest keratocyte density and collagen fibrils.[7, 46–49]

The limitation of this study is the absence of CET and CD measurements in preoperative and in early stages after PRK, but we have compared our findings with the unoperated eyes, even if only of the groups 1 and 2, because it has been demonstrated that the right and left eyes of healthy subjects do not present significant differences in CD[14] and because the groups of 54 and 30 operated eyes compared with unoperated eyes showed similar trend in changes of CET and CD.

The higher values of corneal light backscattering associated with deeper ablations could be influenced also by an old PRK laser technology. It turned to be different respect to last generation excimer laser that presents a higher frequency of treatment, an improved fluence and a higher level of safety (a more precise eye tracking and an efficient suction system that guarantees the constant evacuation of

the fumes during ablation while maintaining the correct distribution of the laser energy on the corneal surface).[50]

The use of mitomycin C demonstrated in previous studies an high and positive impact on the refractive results and in preventing haze formation post PRK[51, 52] although several reports showed the absence of significant differences in haze formation in patients with or without the use of mitomycin C during the follow-up.[53, 54]

In this study, it have been not used corticosteroids or mitomycin C in patients underwent PRK and subjects that presented haze consequent to PRK were excluded.

Lastly, AS-OCT suffers from an inability to discriminate the precorneal tear film, whose thickness was reported to be 4.79 ± 0.88 μm in a previous study[55] and may influence CET assessment.

It would be interesting in future studies to evaluate the relationship between the changes in CD, CET and the corneal stiffness and the refractive changes related to age (such as presbyopia) in these patients.

In conclusion, CET and CD offer valid information that allow a peculiar preoperative assessment and may be used as objective parameters to evaluate corneal response to refractive surgery and to monitor patients over time. Scheimpflug imaging system and AS-OCT represent non-invasive imaging techniques to better understand the pathophysiological events occurring during the PRK follow-up.

Our study provides quantitative and detailed evidences about the changes in corneal epithelium and in anterior CD more than 22 years after PRK showing that the postoperative remodeling process of the anterior corneal structures determined the increase in corneal light backscattering and in CET.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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